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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/529,722	04/19/2000	DAVID J SQUIRRELL	124-765	3335

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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 05/02/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Application No.	Applicant(s)
09/529,722	SQUIRRELL ET AL.
Examiner	**Art Unit**
David J. Steadman	1652

Office Action Summary

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)[x] Responsive to communication(s) filed on *02 April 2003*.

2a)[x] This action is **FINAL**. 2b)[ ] This action is non-final.

3)[ ] Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)[x] Claim(s) *67-106* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)[ ] Claim(s) _____ is/are allowed.

6)[x] Claim(s) *67-106* is/are rejected.

7)[ ] Claim(s) _____ is/are objected to.

8)[ ] Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)[ ] The specification is objected to by the Examiner.

10)[ ] The drawing(s) filed on _____ is/are: a)[ ] accepted or b)[ ] objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)[ ] The proposed drawing correction filed on _____ is: a)[ ] approved b)[ ] disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)[ ] The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)[x] Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)[x] All b)[ ] Some * c)[ ] None of:

1.[ ] Certified copies of the priority documents have been received.

2.[ ] Certified copies of the priority documents have been received in Application No. _____.

3.[x] Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)[ ] Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) [ ] The translation of the foreign language provisional application has been received.

15)[ ] Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) [ ] Notice of References Cited (PTO-892)
2) [ ] Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) [ ] Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____.
4) [ ] Interview Summary (PTO-413) Paper No(s). _____.
5) [ ] Notice of Informal Patent Application (PTO-152)
6) [ ] Other:

DETAILED ACTION

Application Status

[1] A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 04/02/03 has been entered.

[2] Claims 67-106 are pending in the application.

[3] Applicant's cancellation of claims 58-66 and addition of claims 67-106 in Paper No. 28, filed 04/02/03, is acknowledged.

Claim Rejection(s) - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

[4] Claims 67-106 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 67-72 are drawn to methods for producing a polypeptide product by identifying a mutant form of a polypeptide product with increased tolerance to pH or temperature, identifying a mutant form of an undesired protein with decreased tolerance to pH or temperature, transforming a host to express the mutant proteins, culturing said host cell, and recovering the genus of protein products that remain unaffected at a pH or temperature at which the genus of undesired proteins are denatured. Claims 73-82 and 91-97 are drawn to methods for producing a polypeptide product by culturing a host cell that has

been transformed to express a genus of polypeptide products that remain unaffected at a pH or temperature at which an undesired protein is denatured and further transformed to express a genus of undesired polypeptides in mutant form that are denatured under conditions in which a desired polypeptide remains unaffected and optionally wherein the desired polypeptide is luciferase and the undesired protein is adenylate kinase, optionally thermolabile at 37 degrees Celsius, optionally with mutations at positions 87 or 107. Claims 83 (claims 85-87 dependent therefrom), 84, 98 (claims 100-102 dependent therefrom), and 99 are drawn to a recombinant cell expressing a genus of nucleic acids encoding a desired polypeptide that remains unaffected at a temperature at which an undesired protein is denatured and expressing a genus of nucleic acids encoding an undesired polypeptide in a mutant form that is denatured under conditions in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is luciferase and the undesired protein is adenylate kinase. Claims 88 and 89 (claim 90 dependent therefrom) are drawn to a method for producing the recombinant cell of claim 83. Claims 103 and 104 (claim 105 dependent therefrom) are drawn to a method for producing the recombinant cell of claim 98. Claim 106 is drawn to a method for producing a luciferase by culturing a host that has been transformed to express a genus of luciferases that are thermostable at 37 degrees Celsius and a genus of adenylate kinases that are denatured at 37 degrees Celsius. The claims are rejected because the structures of the genera of desired polypeptides, undesired proteins, luciferases, and adenylate kinases have not been adequately described in the specification. The specification teaches only two representative species of such desired polypeptides or protein products that remain unaffected at a temperature at which an undesired protein is denatured or thermostable luciferase proteins, i.e., *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 as described in the cited reference of WO 95/25798 as stated at the bottom of page 8 of the instant specification. Furthermore, the specification teaches only two representative species of undesired proteins in mutant form that are denatured under conditions in which a desired polypeptide remains unaffected or thermolabile adenylate kinases, i.e., *E. coli* adenylate kinase with mutation at position 87 or 107 as set forth at the bottom of page 7 of the specification. The specification fails to describe any other

representative species of desired polypeptides that remain unaffected at a temperature at which an undesired protein is denatured, thermostable luciferase proteins, undesired proteins in mutant form that are denatured under conditions in which a desired polypeptide remains unaffected, or thermolabile adenylate kinases by any identifying characteristics or properties other than the functionality of being a desired polypeptide that remains unaffected at a temperature at which an undesired protein is denatured, a thermostable luciferase protein, an undesired protein in a mutant form that is denatured under conditions in which a desired polypeptide remains unaffected, or a thermolabile adenylate kinase. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

[5] Claims 67-106 are rejected under 35 U.S.C. 112, first paragraph. Regarding claims 67-82, 91-97, and 106, the specification, while being enabling for a method for producing *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 that is substantially free of *E. coli* adenylate kinase with mutation at position 87 or 107, does not reasonably provide enablement for a method of producing any polypeptide product that remains unaffected to any conditions or conditions of temperature or pH at which an undesired protein is denatured that is substantially free of a specific undesired protein in a mutant form that is denatured under any conditions or conditions of temperature or pH in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is any luciferase and the undesired protein is any adenylate kinase, and optionally wherein the adenylate kinase is thermolabile at 37 degrees Celsius, and optionally with mutations at positions 87 or 107. Regarding claims 83 (claims 85-87 dependent therefrom), 84, 88, 89 (claim 90 dependent therefrom), 98 (claims 100-102 dependent therefrom), 99, 103, 104 (claim 105 dependent therefrom), the specification, while being enabling for a recombinant host cell transformed to express a *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 and *E. coli* adenylate kinase with mutation at position 87 or 107 and a method for producing said recombinant host cell, does not reasonably provide enablement for a recombinant host cell expressing all nucleic acids

encoding a desired polypeptide that remains unaffected at a temperature at which an undesired protein is denatured and expressing all nucleic acids encoding an undesired polypeptide in a mutant form that is denatured under conditions in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is any luciferase and the undesired protein is any adenylate kinase and a method for producing said recombinant host cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Undue experimentation would be required for a skilled artisan to make and/or use the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s). The Factors most relevant to the instant rejection are addressed below.

- The claims are overly broad in scope: Regarding claims 83-90 and 98-105, the claims are so broad as to encompass a recombinant host cell expressing all nucleic acids encoding a desired polypeptide that remains unaffected at a temperature at which an undesired protein is denatured and expressing all nucleic acids encoding an undesired polypeptide in a mutant form that is denatured under conditions in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is any luciferase and the undesired protein is any adenylate kinase and a method for producing said recombinant host cell. Regarding claims 67-82, 91-97, and 106, the claims are so broad as to encompass a method of producing any polypeptide product that remains unaffected to any conditions or conditions of temperature or pH at which an undesired protein is denatured that is substantially free of a specific undesired protein in a mutant form that is denatured under any conditions or conditions of temperature or pH in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is any luciferase and the undesired protein is any adenylate kinase, and optionally wherein

the adenylate kinase is thermolabile at 37 degrees Celsius, and optionally with mutations at positions 87 or 10. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the large number of desired polypeptides that remain unaffected at a temperature at which an undesired protein is denatured, thermostable luciferase proteins, undesired proteins in mutant form that are denatured under conditions in which a desired polypeptide remains unaffected, or thermolabile adenylate kinases broadly encompassed by the claims. In this case, claims 83-90 and 98-105 are limited to a recombinant host cell transformed to express a *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 and *E. coli* adenylate kinase with mutation at position 87 or 107 and a method for producing said recombinant host cell and claims 67-82, 91-97, and 106 are limited to a method for producing *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 that is substantially free of *E. coli* adenylate kinase with mutation at position 87 or 107.

- The lack of guidance and working examples: The specification fails to provide guidance that would enable a skilled artisan to make the entire scope of claimed recombinant cells and methods. The specification provides only two working examples of desired polypeptides that remain unaffected at a temperature at which an undesired protein is denatured or thermostable luciferase proteins, i.e., *Photinus pyralis* luciferase with mutation at position 354 or *Luciola* lucifease with mutation at position 356 as described in the cited reference of WO 95/25798 as stated at the bottom of page 8 of the instant specification. Furthermore, the specification provides only two working examples of undesired proteins in mutant form that are denatured under conditions in which a desired polypeptide remains unaffected or thermolabile adenylate kinases, i.e., *E. coli* adenylate kinase with mutation at position 87 or 107 that are denatured at 37 °C as set forth at the bottom of page 7 of the specification. Regarding pH conditions, there is no guidance or working examples in the specification of desired proteins, protein products, or a luciferase that is stable under pH conditions that an undesired protein or adenylate kinase is denatured as the specification provides no indication as to whether the working examples cited above maintain their respective abilities to remain unaffected or become denatured in the presence of any pH conditions. No

other working examples are provided in the specification, and even with a knowledge of the prior art, a skilled artisan would not be able to make the entire scope of claimed recombinant cells and methods.

- The high degree of unpredictability of the art: The ability of any desired protein or luciferase to maintain activity at a temperature or pH at which any undesired protein is denatured is *highly* unpredictable. Similarly, the ability of any undesired protein or adenylate kinase to become denatured or inactivated at a temperature or pH at which any undesired protein is denatured is *highly* unpredictable. The four working examples as taught by applicant are insufficient to provide a skilled artisan with an predictable expectation of success for making the broad scope of the claimed invention. While methods of making mutant proteins and encoding nucleic acid sequences are known, the ability to predict which mutations of any desired protein or luciferase will result in an increased tolerance to temperature or pH or alternatively, the ability to predict which mutations of any undesired protein or adenylate kinase will result in a decreased tolerance to temperature or pH is outside the ability of a skilled artisan, particularly in view of the lack of guidance and working examples provided in the specification. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. In this case, such guidance has not been presented. Even if one were to isolate mutants of any desired protein or luciferase with an increased tolerance to temperature or pH and mutant undesired proteins or adenylate kinase with decreased tolerance to temperature or pH, respectively, it is highly unpredictable whether the combination of proteins will be useful in the claimed methods.
- The amount of experimentation: While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for all mutant proteins with an increased and decreased tolerance to temperature or pH, as encompassed by the instant claims. In view of the broad scope of the claims, the lack of guidance and working examples, and the high degree of unpredictability, the amount of

experimentation required to make the entire scope of claimed recombinant cells and methods would clearly constitute undue experimentation.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejection(s) - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

[6] Claims 67-78 and 80-106 are rejected under 35 U.S.C. 103(a) as being unpatentable over EP 373962 in view of Belinga et al. (*J Chromat A* 695:33-40), Gilles et al. (*Proc Natl Acad Sci, USA* 83:5798-5802), and Kajiyama et al. (*Biochemistry* 32:13795-13799). Claims 67-82, 91-97, and 106 are drawn to methods for producing polypeptide products free of an undesired protein or luciferase free of adenylate kinase, claims 83-87 and 98-102 are drawn to recombinant cells, claims 88-90 and 103-105 are drawn to methods for producing a recombinant cell.

EP 373962 teaches that a thermostable enzyme can be purified from unwanted contaminants that interfere with the intended use of the thermostable enzyme (column 2, lines 38-43) by engineering host cells, including procaryotes such as E. coli (column 4, lines 11-12), to produce a desired enzyme and the desired enzyme can be purified from contaminating proteins by denaturation at a temperature that does not denature the desired thermostable enzyme (column 2, lines 45-49).

Kajiyama et al. teach a vector encoding a mutant thermostable *Luciola cruciata* luciferase (abstract). The mutation resulting in the thermostable luciferase is a substitution of threonine with isoleucine at position 217 (abstract). Kajiyama et al. teach that the encoded wild-type *L. cruciata* luciferase was inactivated by incubation at 50 °C for 40 min, while the thermostable luciferase maintained over 30 % enzymatic activity (page 13796, left column).

Gilles et al. teach thermosensitive mutants of *E. coli* with a mutation in the endogenous *adk* gene encoding adenylate kinase (page 5798, left column). Characterization of the mutant adenylate kinase encoded by the endogenous *E. coli* gene revealed the presence of a substitution of serine for proline at position 87 (page 5798, right column). Gilles et al. teach screening the thermosensitive adenylate kinase against wild-type adenylate kinase indicated that the mutant adenylate kinase is active at 27 °C and is inactivated at 40 °C (page 5798, left column).

Belinga et al. teach that the presence of adenylate kinase interferes with luciferase bioluminescence assays by producing light with nucleotides other than ATP and disclose that it is necessary to remove adenylate kinase during the purification of luciferase (page 33).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of EP 373962, Belinga et al., Kajiyama et al., and Gilles et al. to transform the *E. coli* expressing an endogenous thermolabile adenylate kinase of Gilles or an *E. coli* with a disrupted *adk* gene transformed with a plasmid expressing the mutant adenylate kinase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiyama et al., express the mutant thermostable luciferase and heat the resulting cell or cell extract at a temperature of 40 °C to denature and inactivate the thermolabile adenylate kinase. One would have been motivated to transform the *E. coli* expressing an endogenous thermolabile adenylate kinase of Gilles or an *E. coli* with a disrupted *adk* gene transformed with a plasmid expressing the mutant adenylate kinase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiyama et al., express the mutant thermostable luciferase and heat the resulting cell or cell extract at a temperature of 40 °C to denature and inactivate the thermolabile adenylate kinase because of the teachings of Belinga et al. who taught that is necessary to remove the contaminating adenylate

kinase from luciferase for bioluminescence assays. One would have a reasonable expectation of success for transforming the *E. coli* expressing an endogenous thermolabile adenylate kinase of Gilles or an *E. coli* with a disrupted *adk* gene transformed with a plasmid expressing the mutant adenylate kinase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiyama et al., expressing the mutant thermostable luciferase and heating the resulting cell or cell extract at a temperature of 40 °C to denature and inactivate the thermolabile adenylate kinase because of the results of EP 373962, Kajiyama et al., and Gilles et al. Therefore, claims 67-78, 80-82, 91-97, and 106, drawn to methods for producing polypeptide products free of an undesired protein or luciferase free of adenylate kinase, claims 83-87 and 98-102, drawn to recombinant cells, and claims 88-90 and 103-105, drawn to methods for producing a recombinant cell, would have been obvious to one of ordinary skill in the art.

Conclusion

[7] All claims are rejected.

[8] No claim is in condition for allowance.

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Thursday from 6:30 am to 5:00 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for official papers filed to Group 1600 is (703) 308-4242. Draft or informal FAX communications should be directed to (703) 746-5078. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.
Patent Examiner
Art Unit 1652